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Isolation and antiproliferative activity of *Lotus corniculatus* lectin towards human tumour cell lines

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ABSTRACT

The objective of the study was to investigate the anti cancer activity of a lectin isolated from *Lotus corniculatus* seeds. A tetrameric 70 kDa galactose specific lectin was purified using two step simple purification protocol which involved affinity chromatography on AF-BlueHC650M and gel filtration on Sephadex G-100. The lectin was adsorbed on AF-BlueHC650M and desorbed using 1 M NaCl in the starting buffer. Gel filtration on Sephadex G-100 yielded a major peak absorbance that gave two bands of 15 kDa and 20 kDa in SDS PAGE. Hemagglutination activity was completely preserved, when the temperature was in the range of 20–60 °C. However, drastic reduction in activity occurred at temperatures above 60 °C. Full hemagglutination activity was retained at ambient pH 4–12. Thereafter no activity was observed above pH 13. Hemaglutination of the lectin was inhibited by D-galactose. The lectin showed a strong antiproliferative activity towards human leukemic (THP-1) cancer cells followed by lung cancer (HOP62) cells and HCT116 with an IC_{50} of 39 μg/ml and 50 μg/ml and 60 μg/ml respectively. Flow cytometry analysis showed an increase in the percentage of cells in sub G0G1 phase confirming that *Lotus corniculatus* lectin induced apoptosis. Morphological observations showed that *Lotus corniculatus* lectin (LCL) treated THP-1 cells displayed apparent apoptosis characteristics such as nuclear fragmentation, appearance of membrane enclosed apoptotic bodies and DNA fragmentation. *Lotus corniculatus* lectin (LCL) effectively inhibits the cell migration in a dose dependent manner as indicated by the wound healing assay.

Introduction

Lectins are heterogeneous group of proteins with at least one non catalytic domain that selectively recognises and specifically binds to free sugars present on glycoproteins and glycolipids without altering the structure of the carbohydrates (Lannoo and Van Damme, 2010). Lectins are widely distributed in nature, mainly in the plant kingdom, even though they also occur in other organisms, such as animals and microorganisms (Pustai 1991; Drikarmer and Taylor 1993; Gabius 1997; Wong et al., 2010). Plant lectins have been established to possess remarkable anticancer properties *in vivo*, *in vitro* and in human case studies and have been successfully adopted for alternative cancer therapy (De Mejia and Prisecaru, 2005; Liu et al., 2010; Pusztai et al., 2008). Legume lectins are one of the most comprehensively studied plant lectins for their molecular basis of the protein-carbohydrate interactions for several decades (Damodaran et al., 2008). In recent years, the main interest in this lectin family lay in their prospective application as antitumor agents that could bind specific cancer cell surface glycoconjugates (Ueno et al., 2000) *e.g.* a typical legume lectin with specificity towards sialic acid purified from *Phasoleus coccinus* L.(Phasoleus Multiflorus wild) seeds possess a remarkable antiproliferative activity.

Lotus corniculatus commonly known as Bird's foot trefoil belongs to a genus that contains many dozen of species distributes worldwide *e.g. Lotus aboriginus* (Rosy Bird's foot trefoil), *Lotus angustissimus* (Slender Bird's foot trefoil) and *Lotus argophyllus* (Silver Bird's foot trefoil). *Lotus corniculatus* is a species of the leguminosae family. *Lotus corniculatus* can fix nitrogen through the root nodules making it useful as cover crop. It has been studied for its flavonoid content (Jay and Ibrahim 1986; Sarelli et al., 2003; Reynaud and Lassignol, 2005). *Lotus corniculatus* is known for its medicinal values. The flowers are antispasmodic and sedative (Chiej 1984). The root is carminative and febrifuge (Duke and Ayensu 1985). Although *Lotus corniculatus* belongs to legume family, none of the Lotus species have been investigated for the lectins.

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We therefore isolated and purified a lectin from *Lotus corniculatus* seeds with two simple chromatographic steps. Some physical and chemical properties such as thermo stability, pH stability and carbohydrate specificity of the lectin have been studied. Biological activities of the *Lotus corniculatus* lectin were also studied. The lectin possessed antiproliferative effects on some tumour cell lines by inducing apoptosis and also possesses anti metastatic effects.

Materials and methods

Purification of Lotus corniculatus lectin

The pods of *Lotus corniculatus* were obtained from the surroundings of Kashmir University and authenticated at centre of plant taxonomy (COPT), Department of Botany, Kashmir University, India. The seeds (60 g) were crushed and powdered in liquid nitrogen, dissolved in 300 ml of 10 mM Tris–HCl buffer (pH 7.6), followed by centrifugation at 3000 × *g*, at 4 °C, for 30 min. Ammonium sulphate was added to the supernatant to 40–60% saturation. The precipitate was resuspended in 10 mM Tris–HCl buffer and dialysed extensively overnight at 4 °C. The sample was then adjusted to 10 mM Tris–HCl (pH 7.6) by adding Tris–HCl buffer (1.5 M, pH 7.6). The sample was then applied to AF-BlueHC650M (Toyopearl) column (18 cm × 5 cm) that had been equilibrated with 10 mM Tris–HCl buffer (pH 7.6). Unabsorbed proteins were eluted with the starting buffer. The column was washed with 1 M NaCl in10 mM Tris–HCl buffer to elute the bound protein. The active fractions were pooled, dialysed extensively against double distilled water and concentrated by Ultra filtration using 10 kDa cut-off membrane. The purified lectin was dissolved in sterile distilled water (16 mg/ml). The solution was subjected to Gel filtration on Sephadex G-100 column. A major absorbance peak that contained purified LCL was obtained.

Assay of hemagglutination activity

In a 96-well microtitre U-plate, a serial two fold dilution of the test sample (50 μl) in phosphate buffer saline (PBS) (pH 7.2) was performed. A 2% rabbit red blood cell suspension (50 μl) in PBS was added to the sample. The mixture was incubated at room temperature until the red blood cells in the blank (without protein sample) had fully sedimented and appeared as a red spot at the bottom of the well. Presence of agglutinated red blood cells in the wells indicated hemagglutinating activity. One hemagglutination unit is the reciprocal of the highest dilution of the lectin sample inducing hemagglutination. Specific activity is the number of hemagglutination units per mg protein (Yagi et al., 2002).

Carbohydrate binding specificity

The carbohydrate specificity was investigated by observing the inhibition of the lectin induced hemagglutination by various sugars namely D-glucose, D-galactose, D-mannose, fructose, lactose, maltose, sucrose, ribose, xylose, manitol and sugar derivatives like N-acetyl galactosamine and N-acetyl glucosamine. The inhibition assay was performed in 96-well plate. Different dilutions of the above sugars (final volume 20 μl) were added to the wells in which agglutination was performed. To each dilution, 20 μl of purified lectin was added. The mixture was incubated at room temperature for 1 h after which 80 μl of 2% suspension of erythrocytes was added to each well. The minimum concentrations of each sugar capable of fully inhibiting agglutination after 1 h at room temperature were noted.

Molecular mass determination

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 8% separating gel and a 5% stacking gel was performed. After electrophoresis, the gel was stained with Coomassie brilliant blue and destained with 10% acetic acid overnight (Laemmli and Favre, 1973).

Gel permeation chromatography was carried out using Sephadex G-100 column calibrated with molecular mass standard.

Protein concentration determination

The protein concentration was determined by the method of (Lowry et al., 1951) using BSA as the standard protein.

Effect of temperature, pH on lectin-induced hemagglutination

Thermal stability of LCL was monitored in the range of 10–100 °C by incubating the lectin for 60 min at the respective temperatures, followed by cooling on ice and determination of agglutination activity under standard conditions.

The pH dependence of the lectin was determined by incubating 50 μg of LCL with buffers in different pH:HCl: pH 0–1, glycine/HCl (pH 2–3), 0.05 sodium acetate/acetic acid (pH 4–5), potassium phosphate (pH 6–7), Tris–HCl (pH 8–9) and glycine-NaOH (pH 10–11), pH 11–12 $NaHCO_3$ and pH 13–14, NaOH, for 5 h at 25 °C and pH was adjusted to 7.2 just prior to hemagglutination assay.

Tumour cell lines and culture conditions

Human leukemic cell line (adherent Type) (THP-1), lung cancer cell line (HOP62) and colon cancer cell line (HCT116) were obtained from American Type Culture Collection. All the cell lines were cultured in RPMI 1640 (Sigma) medium which contained FCS 10% (Sigma), 100 U/ml of pencillin (Sigma) and 100 μg/ml of streptomycin (Sigma). Cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. The cells were fed every 2–3 days, and at confluency they were harvested with 0.05% (w/v) trypsin–EDTA (Sigma) and sub-cultured in identical medium.

Sulphorhodamine B (SRB) assay

Human leukemic (Adherent type) (THP-1), Lung cancer (HOP62), and colon (HCT116) cancer cells from American Type Culture Collection were adjusted to a cell density of 5×10^4 cells/ml in RPMI medium. The cells (100 ml) were seeded onto the wells of a 96-well plate and incubated overnight. Different concentrations of *Lotus corniculatus* lectin (100 ml, final concentrations at 30–100 μg/ml) were added to the wells followed by incubation for 48 h. The plates was taken out from the incubator after 48 h of adding the sample. To stop the reaction, 50 μl of chilled 50% TCA (trichloroacetic acid) to each well of the plate was added, making final concentration to 10%. The plate was incubated at 4 °C for 1 h to fix the cells attached to bottom of the wells. The plate was washed 5–6 times with distilled water. Plate was air-dried. 100 μl of SRB dye (0.4% in 1% acetic acid) was added to each well of the plate and the plate left at room temperature for 30 min. The plate was washed with 1% acetic acid after 30 min. The plate was again air-dried. 100 μl of tris buffer (10.5 M) was added to each well. The plate was shaken gently for 10–15 min on a mechanical shaker. The optical density was recorded with ELISA reader at 540 nm.

Measurement of mitochondrial transmembrane potential by rhodamine 123 (Rh-123) staining

THP-1 cells (5×10^5) were seeded onto a 6-well plate and incubated overnight. Different concentrations of *Lotus corniculatus* lectin (30–100 μg/ml) were added to the cells and incubated for 24 h. The cells were trypsinized and centrifuged down at $1500 \times g$ for 5 min. Then the cells were washed with PBS and again centrifuged at $1500 \times g$ for 5 min, thrice. The cell pellets were resuspended in RPMI medium (500 μl) containing 10 μM Rh-123 dye, and incubated at 37 °C in dark. The cells were analysed using a FACs flow cytometer for detecting the mitochondrial depolarization patterns.

Cell cycle distribution analysis

After 24 h of incubation, to exponentially growing THP-1 cells (1×10^5) 30–100 μg/ml of LCL was added and incubated further for 24 h. Control cells were devoid of LCL. Both control and LCL treated cells were harvested by trypsin treatment, washed in cold PBS, fixed with 70% ethanol for about 16 h at 4 °C and again washed thrice with cold PBS. In order to remove RNA, the cells were treated with 500 μl A of RNase (100 μg/ml) for 30 min at 37 °C followed by staining with 100 μl of PI (50 μg/ml) at 4 °C for 5 min before analysing by flow cytometry (BD Biosciences)

Observation of cell morphologic changes and nuclear damage

Exponentially growing THP-1 cells (1×10^5 cells/ml) were incubated for 24 h with 30–100 μg/ml of LCL. Apoptotic nuclear morphology was visualised by DAPI staining technique. Cells were fixed with 3.7% of para-formaldehyde for 10 min at room temperature, washed three times with PBS and immersed in 0.1% of Triton X-100 for 2 min. Thus para-formaldehyde-fixed cells were stained using DAPI (10 μg/ml) under dark for 10 min. After three times of washing with PBS the cells were observed under ultraviolet illumination with a confocal microscope (Olympus).

DNA fragmentation assay

DNA fragmentation was carried out as described by (Lin et al., 2003) with slight variations. Five hundred microliters of (5×10^6) treated cells were lysed in 55 ml lysis buffer [1 M Tris–HCl (pH 8.0), 0.5 M EDTA, and 100% Triton X-100] and incubated at 4 °C for 30 min. DNA was extracted from the supernatant with an equal volume of phenol/choloroform/isoamyl alcohol (25:24:1). Samples were spun and the upper aqueous layer transferred to a new tube, to which an equal volume of ice-cold 100% ethanol and 1/10 volume of 3 M sodium acetate (pH 5.2) were added and incubated at −20 °C overnight. After spinning the sample, supernatant was decanted, pellet air dried, and then dissolved in deionised water-RNase solution [10 mg/ml RNase I] and incubated at 37 °C for 30 min. Equal amounts of DNA (10 mg/well) were electrophoresed in 1% agarose gel impregnated with ethidium bromide at 5 V for the first 5 min and increased to 100 V for 1 h. After electrophoresis was complete, gel was observed in gel doc system (Bio-Rad).

Wound healing assay

THP-1 cells 1×10^5 cells/ml/well were seeded in 6-well plate. When the confluency reached 90% wounds were created with the help of a 200-μl pipette tip. The cells were then rinsed with medium to remove any free-floating cells and debris. Medium containing various concentrations of LCL (30–100 μg/ml) was then added and culture plate was incubated at 37 °C. Wound healing was observed after 24 h within the scrape line and representative scrape lines were photographed.

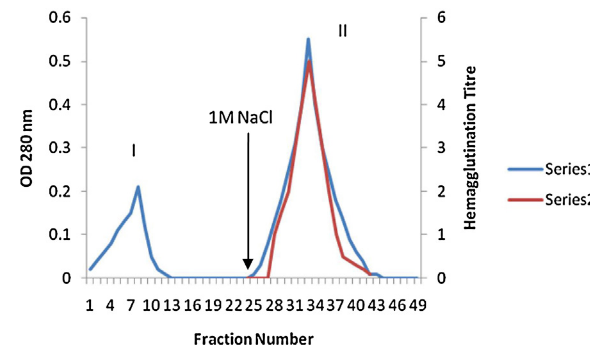

Fig. 1. Affinity purification of LCL from crude extract of its seeds on AF-BlueHC650M (18 cm × 5 cm). About 35 mg of crude extract of LCL was applied to the column, pre-equillibrated with 10 mM Tris–HCl buffer, pH 7.6. Bound protein was eluted with sodium chloride (1 M) in 5 ml fractions at a flow rate of 30 ml/h. Only peak II showed agglutination activity. Series 1 represents Absorbance at 280 nm and Series 2 represents hemagglutination titre.

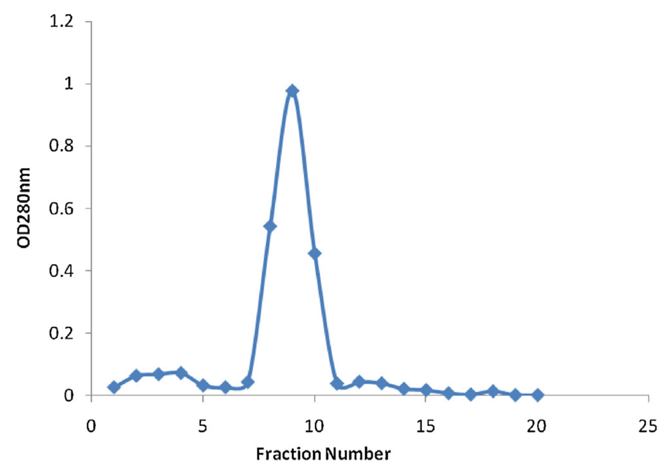

Fig. 2. Gel filtration of the active fractions from the AF-BlueHC650M column on Sephadex G-100 column. Eluent was PBS, pH 7.2. The flow rate was 20 ml/h and fraction size was 3 ml.

Statistical analysis

Results are expressed as the means and standard deviations of triplicate measurements. Each experiment was performed at least thrice. Statistical comparisons were made by student's *t*-test and $p < 0.05$ was considered statistically significant.

Results

Purification of LCL by affinity chromatography on AF-BlueHC650M

Lectin (LCL) extracted from the seeds of *Lotus corniculatus* was purified to homogeneity by affinity chromatography using AF-BlueHC650M column (Fig. 1) and Gel filtration chromatography on Sephadex G-100 (Fig. 2). The summary of purification steps for LCL is presented in Table 1. The lectin was purified 6.54 folds with a recovery of 27.3% activity.

Electrophoretic and chromatographic analysis

LCL showed two bands (15 kDa and 20 kDa) respectively on SDS-PAGE (Fig. 3). It was eluted in about 10th fraction in Sephadex G-100 column. Based on the calibration curve of the column, LCL

Table 1
Summary of purification steps for LCL.

Purification step	Total hemagglutination activity (HU)	Total protein (mg)	Specific activity (HU/mg)	Purification fold	Recovery of activity (%)
Crude	74350	98	774.5	1	100
Ammonium sulphate (40–60%)	63486	38	1670	2.16	85.39
AF-BlueC650M	44320	16	2770	3.58	59.6
Sephadex G-100	20240	4	5060	6.54	27.3

has a molecular size of around 70 kDa. This showed that LCL is a tetrameric protein consisting of two 15 kDa subunits and two 20 kDa subunits.

Effect of pH and temperature on lectin activity

LCL had a moderate thermostability and pH stability. Hemagglutination activity of LCL was completely retained upto 60 °C but further increase in temperature caused abrupt disruption of the activity (Fig. 4). Also complete hemaglutination activity of LCL was observed at pH 4–12, only minimal activity remained at pH 0–2 and whole of the activity was lost above pH 13 (Fig. 5).

Sugar specificity

LCL was a D-galactose binding lectin. The presence of 25 mM Galactose completely inhibited the hemagglutinating activity of the lectin, other sugars were not able to inhibit LCL activity even at a concentration of 200 mM (Table 2).

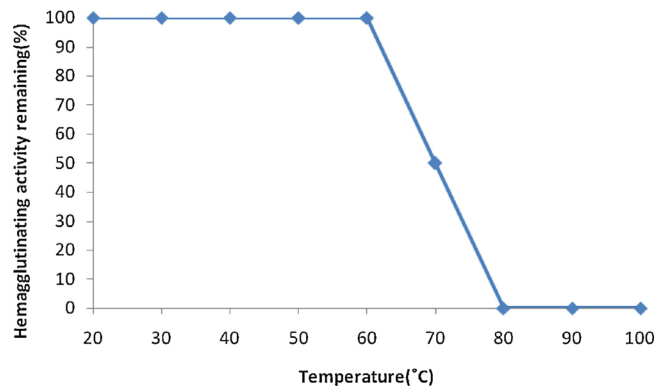

Fig. 4. Effect of temperature on lectin activity of LCL. The lectin was active at 60 °C. Hemagglutination % represents the activity left after treatment at various temperatures ranging from 20 °C to 100 °C.

Effect of LCL on proliferation of tumour cells

The antiproliferative effect of LCL on THP-1, HOP62 and HCT-116 cell lines is shown in Fig. 6. LCL was found to have consider-

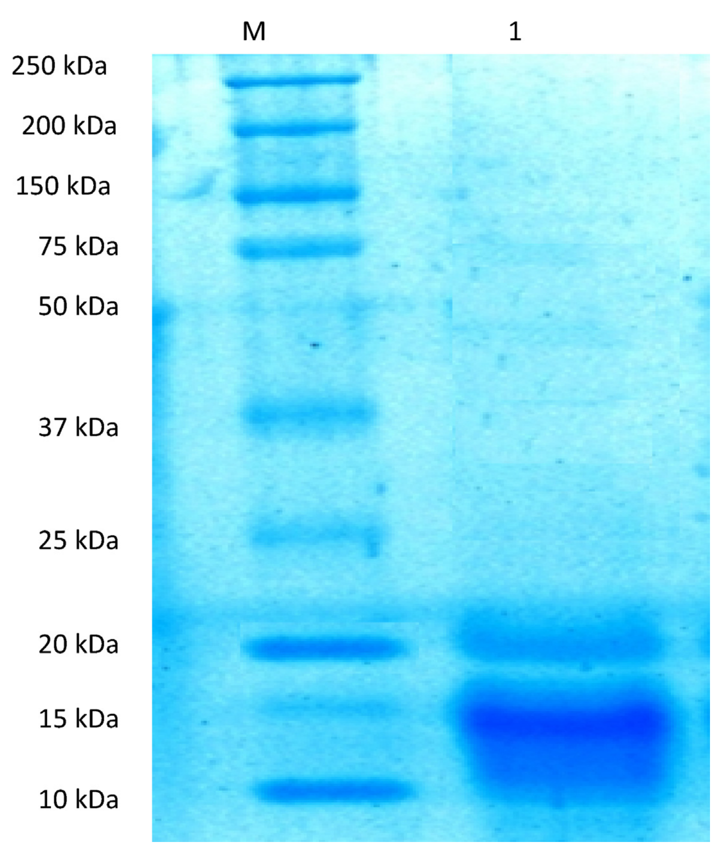

Fig. 3. SDS-Polyacrylamide Gel Electrophoresis pattern of *Lotus corniculatus* lectin (LCL). About 40 μg of LCL was electrophoresed on 8% polyacrylamide gel in presence of 0.1% SDS. Tris–glycine buffer pH 8.3 was used. Current was 8 mA per well. The staining reagent used was Coomassie brilliant blue G-250. M – represents molecular mass marker proteins (Bio-Rad) and 1 represents purified lectin.

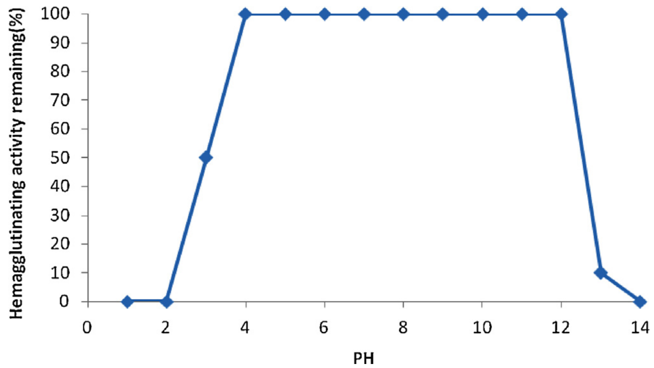

Fig. 5. Effect of pH on LCL activity. The lectin was stable in the pH range of 4–12.

Table 2
Effect of different carbohydrates on hemagglutinating activity of *Lotus corniculatus* lectin (LCL).

Carbohydrate	Inhibitory concentration (mM)[a]
Glucose	NI[b]
Galactose	25
Mannose	NI
Fructose	NI
Lactose	NI
Glucosamine	NI
Galactosamine	NI
Fucose	NI
Arabinose	NI
Melibiose	NI
Glucuronic acid	NI
Xylose	NI
Mannosamine	NI
Mannitol	NI

[a] All the sugars except galactose, could not inhibit the hemagglutination activity even at 200 mM.
[b] Not inhibited.

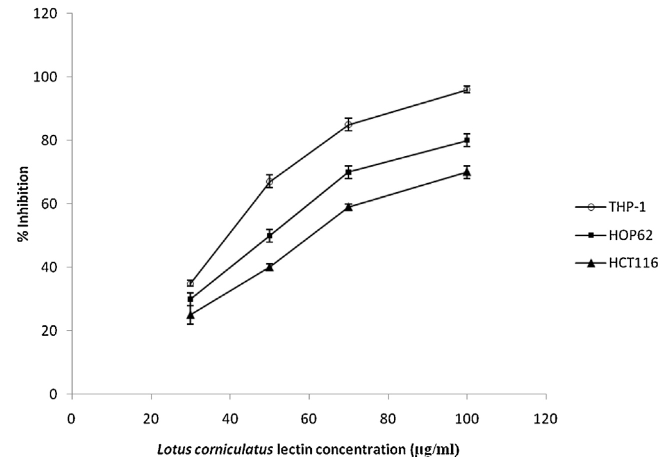

Fig. 6. Result of SRB assay on different cell lines. The cells were treated with LCL for 48 h. LCL exerted strong anti-proliferative activity on THP-1 cells, followed by HOP62 and HCT116. Results represent mean ± SD (n = 3).

able inhibitory effect on the proliferation of THP-1, HOP62 and HCT116 with an IC_{50} of 39 µg/ml, 50 µg/ml and 60 µg/ml respectively. As maximum inhibition of proliferation (95%) was observed in THP-1 cell line, further studies were done using THP-1 cell line.

Effect of LCL on tumour cell cycle

Healthy THP-1 cells exhibited normal cell cycle characteristics (G1/G0 and G2/M phases); the percentage of cells in sub G0/G1 phase was 5.6%. After treatment of THP-1 cells with low concentration of LCL (30 µg/ml), there was a mild increase. *i.e.* 7.2% of THP-1 cells were in sub G0/G1 phase. However, when LCL concentration

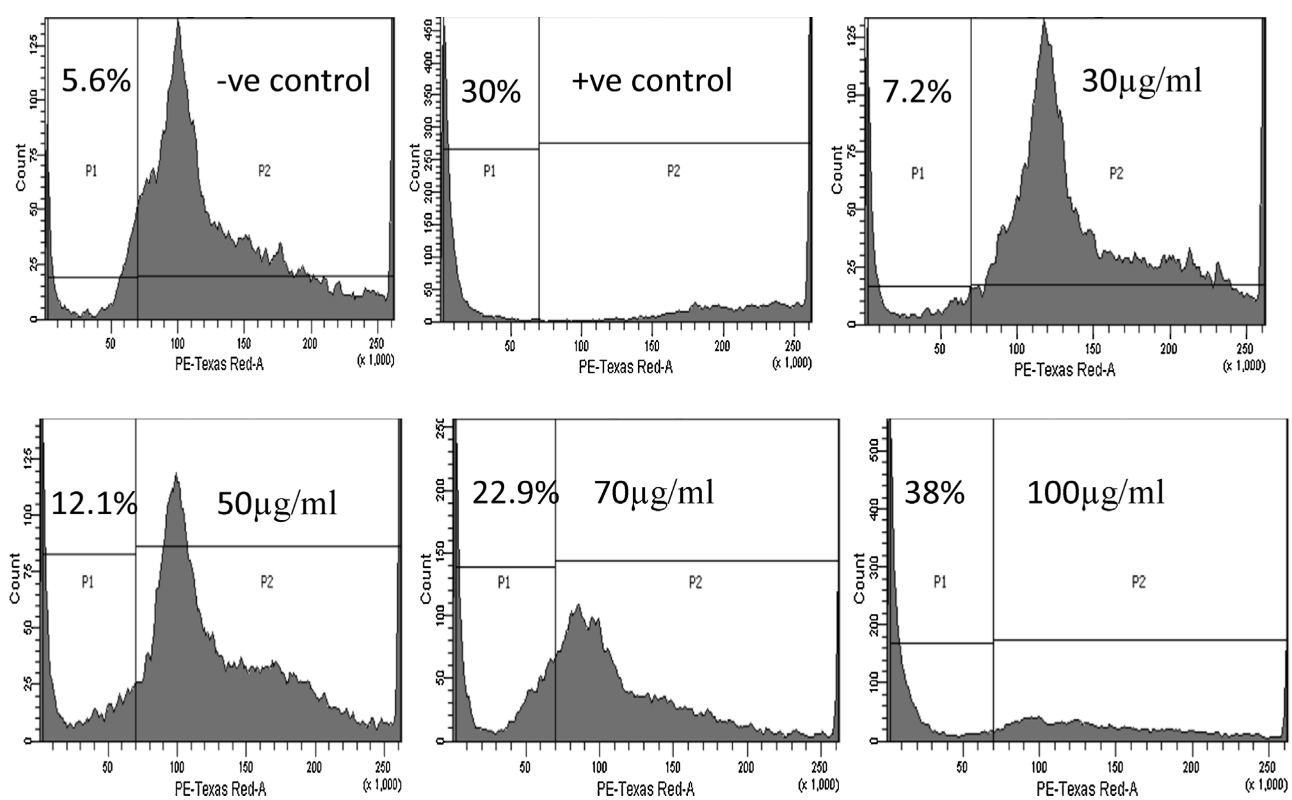

Fig. 7. DNA cell cycle analysis of LCL treated THP-1 cells for 24 h. Cells were exposed to different concentrations (30–100 µg/ml) of LCL for 24 h and stained with PI to determine DNA fluorescence and cell cycle phase distribution. The Data are representative of three independent experiments.

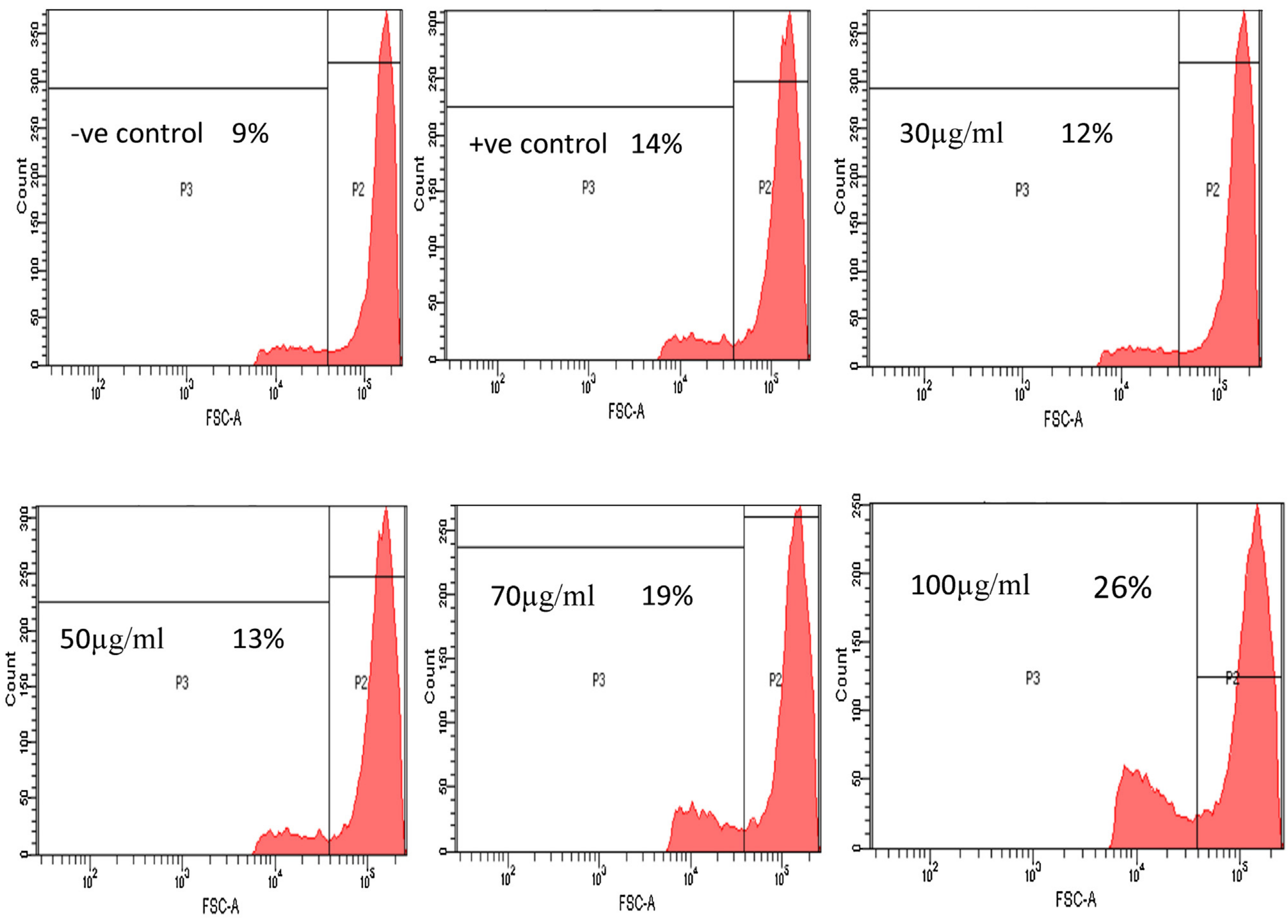

Fig. 8. Rh-123 Staining. LCL induced loss of mitochondrial membrane potential (ΔΨm). THp-1 cells incubated with different concentrations (30–100 μg/ml) of LCL for 24 h. Thereafter, cells were stained with Rhodamine-123 (Rh-123) for 1 h and analysed in FL-1 vs. FL-2 channels of flow cytometer. Data is representative of three independent experiments.

was further increased, the percentage of THP-1 cells in sub G0G1 phase had significantly increased to 38% (Fig. 7). These results indicate that with the increase in concentration of LCL (30–100 μg/ml), the cell cycle of THP-1 cells notably altered, that the sub G0G1 ratio gradually increased, suggesting LCL induces THP-1 cell apoptosis.

Effect of LCL on mitochondrial membrane potential

Loss of mitochondrial membrane potential has been linked to the initiation and activation of apoptotic process in cells (Reed 1995; Hockenbery et al., 1990). In Rh-123 staining of LCL treated THP-1 cells (Fig. 8), the increase in concentration of LCL caused majority of the cells to experience mitochondrial depolarization indicating that the cells were undergoing cell death.

Effect of LCL on cell morphology and nuclear integrity

When THP-1 cells were cultured with LCL for 24 h, morphologic changes were observed and confirmed by DAPI staining. In the control group, the nuclei were round and homogenously stained (Fig. 9), whereas the LCL treated cells showed apoptotic body and condensed chromatin formation.

DNA ladder assay of apoptosis in THP-1 cells

To investigate the apoptosis inducing activity of LCL on THP-1 cells, the integrity of the genomic DNA of lectin treated cells were analysed by agarose gel electrophoresis. As shown in Fig. 10, there is no degradation of genomic DNA extracted from THP-1 cells of Negative control group (Lane 2), whereas there is faint ladder like pattern in THP-1 cells treated at 50 μg/ml, 70 μg/ml and 100 μg/ml, respectively, indicating therefore the occurrence of apoptosis.

LCL inhibited migration of THP-1 cells

The effect of LCL on THP-1 cell migration was determined by scratch motility or wound healing assay. In the scratch motility assay, untreated THP-1 cells exhibited a complete wound closure activity after 24 h (Fig. 11). In contrary; the LCL treated cells showed only limited wound closure at the end of its incubation time by migrating into the denuded zone. LCL showed a reduction in wound closure in a dose dependent manner *i.e.* (30–100 μg/ml).

Discussion

It is now widely acknowledged that plant lectins have an immense potential in treating, preventing and helping to diagnose various chronic diseases including cancer (De Mejia and Prisecaru,

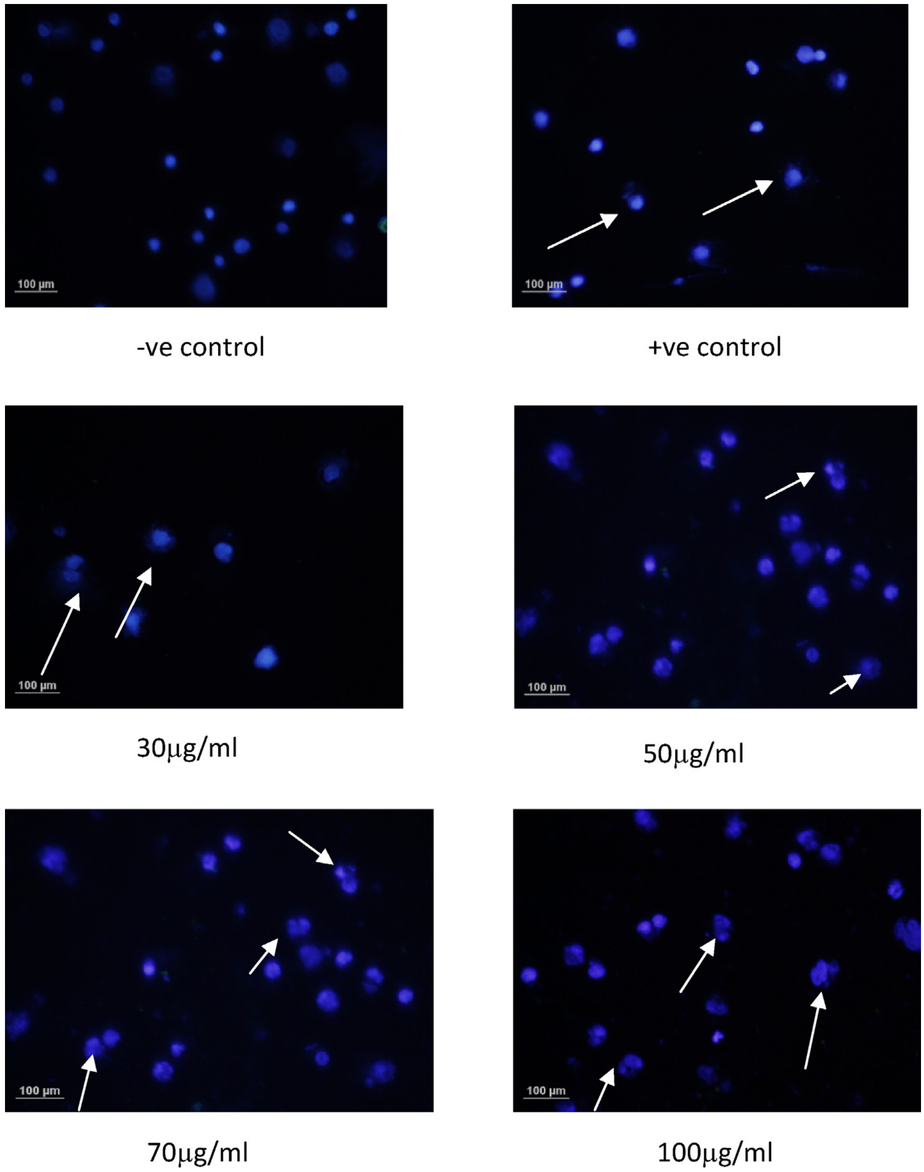

Fig. 9. Morphology observation in THP-1 cells as indicated by DAPI staining. Cells were incubated in absence or presence of different concentrations (30–100 μg/ml) of LCL for 24 h. Arrows indicate apoptotic features (chromatin condensation and nuclear fragmentation). Results are representative of triplicate experiments.

2005). In the present study we have found that LCL possesses an obvious cytotoxicity against THP-1 cancer cells and inhibits migration of the THP-1 cells in a dose dependent manner. LCL was purified from the seed extract after only two simple chromatographic steps.

The molecular weight of LCL is 70 kDa, similar to other reported lectins like *Dioscorea opposita* lectin (Chan and Ng, 2013). LCL is a heterotetramer composed of two subunits of 15 kDa and two subunits of 20 kDa respectively and may be regarded as DBL (*Dolichos biflorus* lectin) type, which is a heterotetramer (Buts et al., 2001). Lectins can be classified according to their carbohydrate binding specificity, such as galactose binding, glucose binding, mannose binding, *etc.* LCL is a D-galactose specific lectin. LCL was demonstrated to possess remarkable antiproliferative effect towards THP-1 cells. A number of lectins have been reported to possess antiproliferative activity towards different cell lines (Dhuna et al., 2007; Xia and Ng, 2006; Fang et al., 2010). The mechanism of action of legume lectins is different from that of Ribosome inactivating proteins (RIP sII) *e.g.*, *Sophora flavescens* lectin induces apoptosis in HeLa cells in a caspase dependent manner (Liu et al., 2008), *Astragalus membranaceus* lectin also induces apoptosis in leukemia cells in a caspase dependent manner (Huang et al., 2011). While as, RIPsII like Mistletoe lectin (MLII) exerts anti-cancer effect by activating extra-cellular signal regulated kinases and P_{38} mitogen activated protein kinase (MAPK) (Heike et al., 1999). The cell cycle dynamics of different phase is regulated due to different factors and different mechanisms *e.g.* many anticancer agents arrest the cell cycle at the sub G0/G1, S, G2/M phase and then induce apoptotic cell death (Deepa et al., 2012; Yan et al., 2009; Lam and Ng, 2010). The results suggest that LCL induces apoptosis and inhibits cell proliferation in THP-1 cells via sub G0–G1 phase arrest of the cell cycle. Apoptosis or programmed cell death is an intrinsic cell suicidal mechanism that plays an imperative role in the maintenance of healthy tissues. Thus, searching for agents which prompt apoptosis of tumour cells has become an attractive strategy in anti-cancer drug discovery (Huang et al., 2011) Apoptosis is characterised morphologically by loss of cell membrane asymmetry, cell shrinkage, apoptotic body formation and condensation of the

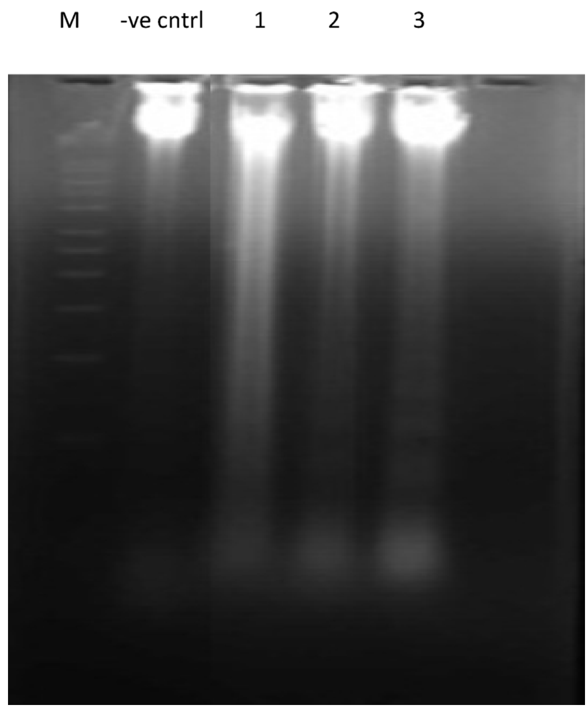

Fig. 10. DNA fragmentation of THP-1 cells treated with LCL. M – represents molecular weight marker, –ve control represents untreated cells, Lane 1–3 represents increasing concentration of LCL (50 μg/ml,70 μg/ml and 100 μg/ml) respectively.

chromatin and DNA fragmentation (Huang et al., 2011; Hengartner, 2000; Liu et al., 2008). DNA condensation and the formation of apoptotic bodies in LCL treated cells were observed with DAPI staining (Fig. 9). The mitochondrial apoptotic pathway is one of the main routes to initiate apoptosis (Kuo et al., 2010). Different stimuli cause changes in the inner mitochondrial membrane leading to the opening of the mitochondrial permeability transition pore, loss of the mitochondrial membrane potential (Saelens et al., 2004) and pro-apoptotic protein release from the intermembrane space into the cytosol (Borutaite, 2010; Singh et al., 2011). Our studies demonstrated that treatment with LCL increased mitochondrial membrane potential loss in a dose dependent manner, which may indicate cell death by apoptosis in THP-1 cells (Fig. 8). Some lectins such as Con A, BKBL, yam, may cause disruption of the mitochondrial membrane potential as an event associated with apoptosis (Liu et al., 2009; Chan et al., 2012; Chan and Ng, 2013). The most distinct biochemical characteristic of apoptosis is the activation of endogenous Ca^{2+}/Mg^{2+} dependent endonuclease mediated cleavage of nucleosomes to generate oligonucleotide fragments with about 180–200 bp length or their polymers (Wyllie, 1997). These fragments appear as DNA ladder on an agarose gel as shown in Fig. 10, instead of a randomised DNA breakdown which is observed as a smear in case of necrosis. Cell motility and adhesion are the critical processes in metastasis once the tumour cells invade blood or lymph capillaries successfully. Several factors contribute to cancer cell motility, including insulin-like growth factor II (IGF-II) and autotoxin (ATX), hepatocyte growth factor/scatter factor (HGF/SF), *etc.* (Noble et al., 2003; Gaetano et al., 2009; Martin and Jiang, 2010). LCL effectively inhibits the migration of THP-1 cells (Fig. 11) but whether LCL inhibits the migration of THP-1 cells by affecting the activity of these above mentioned molecules needs to be studied separately.

Conclusion

In conclusion, our observations indicated that LCL effectively caused cytotoxicity of THP-1 cells. In addition, it has the capability to exert inhibitory effect on migration of THP-1 cells, which is a crucial step in cancer metastasis. Nevertheless, a better understanding on the detailed mechanism of how LCL exerts its anti apoptotic and antimetastatic activity would be indispensable, and hence warrants further investigation.

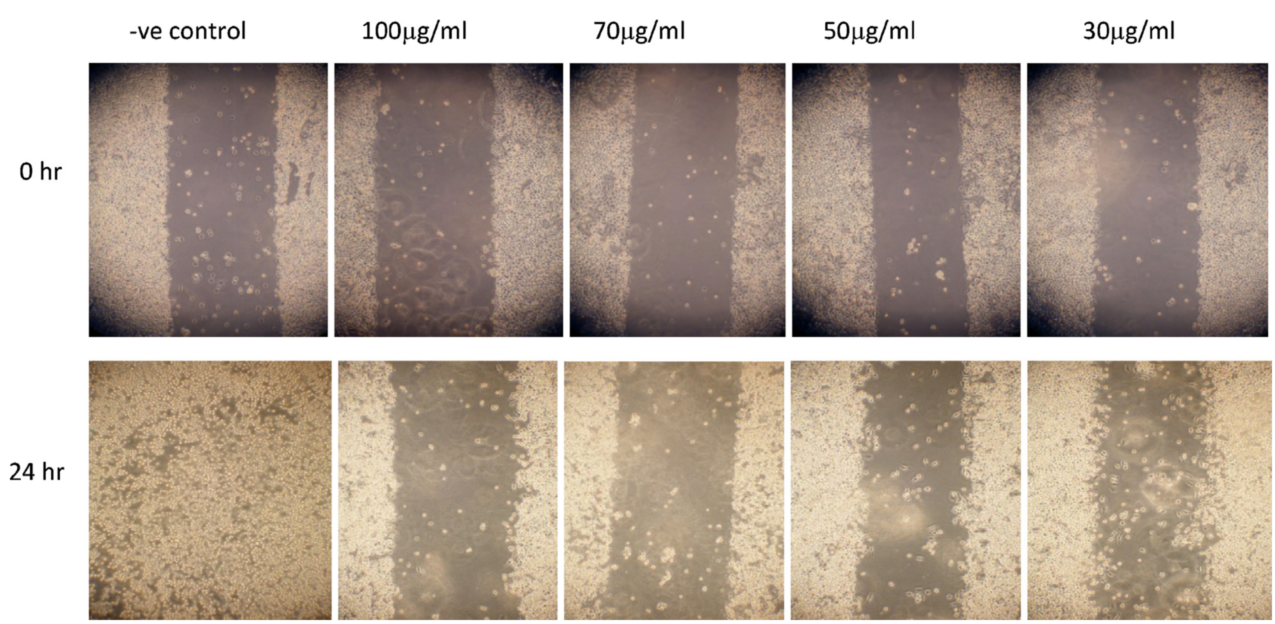

Fig. 11. Wound closure activity of treated THP-1 cells after 24 h with increasing concentration of LCL (30–100 μg/ml). Representative photographs of wounded THP-1 cell monolayered treated with LCL at 0 and 24 h. Untreated control had completely healed. Typical result from three independent experiments is shown.

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